

Protective Activity of Ethanolic Extract of *Curcuma Longa* Root Against Cadmium-Induced Cerebellar Toxicity in Rats

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Submitted: 10 February, 2022. Published: July 2022

ABSTRACT

Background: Due to the rise in industrialization in recent decades, humans are at a high risk of cadmium exposure. Cadmium toxicity, mediated by oxidative stress, accumulates in the body causing organ and central nervous system damage. *Curcuma longa* is a naturally occurring plant reported to possess potent antioxidant properties. Accordingly, this study was designed to investigate its activity against cadmium-induced cerebellar damage in Wistar rats. **Methodology:** Thirty adult Wistar rats were randomized into six groups of five rats each viz: A: Control, distilled water; B: Cadmium (Cd; 5 mg/kg body wt.); C: Ethanolic *Curcuma longa* root extract (ECLRE; 100 mg/kg body wt.) + Cd (5 mg/kg); D: ECLRE (200 mg/kg body wt.) + Cd (5 mg/kg); E: ECLRE (100 mg/kg body wt.); F: ECLRE (200 mg/kg body wt.). All administrations were through an orogastric tube for 21 days. Thereafter, body and brain weight, neurobehavioural, biochemical and histological alterations were investigated. **Results:** Findings revealed a significant decrease in the brain and body weight, impaired locomotion behaviour, increased lipid peroxidation, decreased SOD, CAT and GPx activities as well as loss of Purkinje cells in the Purkinje layer and dissociation of the Purkinje layer from the granular layer in the cerebellum of rats treated with cadmium alone. However, pre-treatment with ECLRE protected the rats against the alterations induced by cadmium. **Conclusion:** ECLRE attenuated oxidative damage induced by cadmium in experimental rats. These findings suggest that *Curcuma longa* may be a promising neuroprotective agent useful in the search for therapeutic options against cadmium-induced neurotoxicity.

Keywords: *Curcuma longa*, Cadmium, Neurotoxicity, Cerebellum, Wistar rats



Access to the article

Website: www.jmbsr.com.ng

doi: [10.5281/zenodo.6450281](https://doi.org/10.5281/zenodo.6450281)

How to cite this article

Enogieru AB, Adedeji OI. Protective activity of Ethanolic Extract of *Curcuma longa* root against Cadmium-induced Cerebellar Toxicity in Rats. *J Med & Bas Sci Res.* 2022;3(2):19-32. DOI: [10.5281/zenodo.6450281](https://doi.org/10.5281/zenodo.6450281)

INTRODUCTION

Cadmium is a naturally occurring soft, malleable, ductile and silvery-white divalent metal that is contained in cigarettes and batteries, fossil fuels, cement and fertilizers^[1].

Due to the high rate of industrialization and the presence of cadmium in certain foodstuffs like mushrooms, shellfish and cocoa powder, human exposure to cadmium either through ingestion or inhalation is almost inevitable. Cadmium is absorbed rapidly and accumulates in various organs and systems of the body. At the cellular level, cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities^[2].

Exposure to cadmium induces oxidative stress through excessive production of reactive oxygen species (ROS) thereby overwhelming the natural antioxidant defences in the body and affecting several neurobiological processes^[3]. Reports indicate that oxidative stress can be attenuated by increasing the body's antioxidants to combat the effects of these free radicals^[4]. Accumulating evidence suggests that most medicinal drugs have adverse effects on the body, thus the use of herbs and medicinal plants for the treatment of diseases have been encouraged. Some of these medicinal plants/herbs include *Carpobrotus edulis*, *Sutherlandia frutescens* and *Crossyne guttata*^[5-7]. *Curcuma longa* (turmeric) is a natural plant that possesses numerous medicinal properties in the traditional system of medicine^[8]. It is a flowering plant of the ginger family that grows in Nigeria and also belongs to the group of oldest cultivated spice plants in the South-East Asian countries^[9, 10]. Throughout Asia, *Curcuma longa* is utilized as a food additive and traditional herbal medicine. Epidemiological evidence supports a positive

correlation between *Curcuma longa* and better cognitive function in the elderly^[11]. Also, research evidence indicates that *Curcuma longa* is neuroprotective against Alzheimer's disease (AD), depression and other neurodegenerative and neuropsychiatric disorders primarily through its anti-inflammatory and antioxidant properties^[12]. A variety of constituents have been identified in *Curcuma longa*. Polyphenolic curcuminoids are the primary group of *Curcuma longa's* bioactive compounds which include curcumin, bisdemethoxycurcumin, demethoxycurcumin, and cyclocurcumin. Although curcumin is the most studied constituent of *Curcuma longa*, others include sesquiterpenes, resins, proteins, caffeic acid and carbohydrates^[12].

Curcumin, a highly pleiotropic molecule, is the major yellow polyphenol present in the rhizomes of *Curcuma longa*. It is available in several forms including capsules, tablets, ointments and energy drinks^[13]. Studies indicate that curcumin binds with redox-active metals and has a potent neuroprotective potential^[14].

There is evidence to show that some populations with a high intake of curcumin-rich diet exhibit low incidence of AD and high cognitive ability attributable to the use of curry, a routine food spice that contains *Curcuma longa/curcumin*^[15]. Other pharmacological activities of curcumin include antimicrobial, anti-inflammatory, antiplatelet, hypoglycaemic, antiangiogenic, antioxidant, antimutagenic and wound healing effects^[12]. Curcuminoids, natural polyphenol compounds derived from *Curcuma longa*, have been approved by the US Food and Drug Administration as "Generally Recognized As Safe". Also, good tolerability and safety profiles have been revealed

by clinical trials at varying doses^[13,16,17]. Although there are numerous beneficial properties of *Curcuma longa*, there is a paucity of research evidence to demonstrate the protective activity of *Curcuma longa* on cadmium-induced neurotoxicity. Accordingly, this study is aimed at investigating the protective activity of ethanolic *Curcuma longa* root extract on cadmium-induced cerebellar toxicity in adult Wistar rats. The results from this study will provide the first research evidence on the neuroprotective activity of *Curcuma longa* in Wistar rats exposed to cadmium.

MATERIALS AND METHODS

Preparation of Plant Material

Roots of *Curcuma longa* were bought from a nearby market and identified at the Department of Plant Biology and Biotechnology, University of Benin, Edo state with herbarium number UBH-C397. The roots were chopped into smaller pieces, air-dried on a slab in the laboratory and 3kg of the sample was pulverized using an industrial grinding machine. The sample was soaked in 7.5 litres of absolute ethanol of analytical grade and kept in a dark cupboard for 72 hours with intermittent stirring before filtering with a cheesecloth. The filtrate obtained was evaporated at 60°C using a vacuum rotary evaporator and freeze-dried using a vacuum freeze-drier. Preservation was through refrigeration at 4°C until needed and subsequently, the crude extract was dissolved in distilled water to make a concentration of 100 mg/ml from which different doses of 100 and 200 mg/kg body weight by oral route were reconstituted.

Preliminary phytochemical screening

Phytochemical evaluation of *Curcuma longa* rhizomes was carried out using standard methods

previously reported^[18,19].

Chemicals and reagents

Normal saline was manufactured by Unique Pharmaceuticals, Sango-Otta, Nigeria and CdCl₂ 98% purity by Loba Chemie Pvt. Ltd, Mumbai, India. Other reagents were all of the analytical grades.

Ethical approval

This study was reviewed and approved by the Research Ethics Committee of the College of Medical Sciences, University of Benin, with the number CMS|REC|2021|171.

Care and Management of Animals

The experiments were performed at the Anatomy Department, School of Basic Medical Sciences, University of Benin. Animals were gotten from the breeding colony of the Department of Anatomy and were fed with top feeds growers mash (manufactured by Premier feed mills Co. Ltd, 1 Eagle Flour Road, Lagos/Ibadan expressway Toll point, Ibadan, Oyo State, Nigeria) and had access to water throughout the entire study period. They were weighed weekly before commencement and throughout the experiment using a digital weighing scale calibrated in grams and recorded to the nearest whole number. The animals received humane care following the principle of humane care and the use of laboratory animals.

Research design

Following two weeks' acclimatization, thirty (30) adult rats, weighing between 130g - 180g, were divided into six groups of five animals each (Table 1).

Neurobehavioral Activity

To assess the effects of treatments on locomotion and exploration, the open field test (OFT) was carried out. The open-field test is a widely used model of exploratory behaviour and general locomotor activity of rodents. This OFT was performed as previously reported^[20] with each rat placed in a 72cm by 72 cm square box with lines on its floor dividing it into 18cm by 18 cm square. Rats were individually placed in the centre of the open field and behaviors (ambulation, immobility, rearing and grooming) in the arena were recorded by an overhead Digital Video Camera Camcorder (LEBAIQI 2.7 Inch Full HD 720P).

Determination of relative brain weight

Following the completion of the OFT, rats were sacrificed by cervical dislocation and the skulls were opened to access the brain. Thereafter, the brains of the rats were weighed, and the cerebellum was separated and processed for biochemical and histological investigation. To decrease the individual bodyweight differences, the relative weight of the brain (%) was expressed as a percentage of the final body weight at sacrifice^[21].

Evaluation of biochemical parameters

The cerebellum was homogenized in ice-cold 20 mM Tris-HCl buffer (pH 7.4), and the homogenates were thereafter centrifuged at 10,000 g for 10 min at 4°C^[22]. The supernatants were collected and evaluated for Superoxide Dismutase^[23], Catalase^[24], Malondialdehyde^[25] and Glutathione Peroxidase(GPx)^[26].

Histological evaluation

Following harvesting of the cerebellum from experimental rats, they were fixed in Bouin's fluid for seventy-two hours. Thereafter, samples were

processed through the paraffin wax embedding method as previously reported^[27]. The Haematoxylin and Eosin staining method described by Drury and Wallington was also performed^[27]. The processed slides were captured with a LABO[®] research trinocular microscope (Labo Microsystems GmbH, Germany) on which were mounted an Omax 9.0MP USB Digital Microscope Camera (made in Korea) domiciled in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city.

Statistical analysis

All data were analysed using GraphPad Prism Software V7 (www.graphpad.com/scientific-software/prism/). Values are presented as mean \pm standard error of mean (SEM), and One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test was used to determine statistical significance ($p < 0.05$).

RESULTS

Phytochemical screening

Results of the phytochemical screening are shown in Table 2. The ethanolic *Curcuma longa* root extract was found to contain tannins, phenols, saponins, cardiac glycosides, flavonoids, terpenes, steroids and alkaloids.

Effects of treatments on Brain and Body Weight

Table 3 shows the body weight, absolute whole brain, and relative brain weights of control and treatment groups after 21 days. For final body weight, there was a decrease ($P < 0.05$) in the cadmium-treated alone group (Cd) when compared to control. An increase was observed in the rats pre-treated with *Curcuma longa* (Cd + CL1 & Cd + CL2) when compared to cadmium-treated

alone group, although this was insignificant ($P>0.05$). For absolute whole brain weight, a significant decrease ($P<0.05$) was observed in cadmium-treated alone group when compared to control. An increase was also observed in groups pre-treated with *Curcuma longa* when compared to cadmium-treated alone group, however, this was

also insignificant ($P>0.05$). For relative brain weights, no significant difference was observed across experimental groups ($P>0.05$).

Effect of treatment on Neurobehavioural activity

Figure 1 shows the open field test of control and

Table 1: Treatment regimen across experimental groups

GROUPS	
A – Control	served as control, received distilled water only
B – Cd	received Cadmium (5 mg/kg body wt.)
C - Cd + CL1	received 1 00 mg/kg body wt. of ethanolic <i>Curcuma longa</i> root extract + Cadmium (5 mg/kg body wt.)
D - Cd + CL2	received 200 mg/kg body wt. of ethanolic <i>Curcuma longa</i> root extract + Cadmium (5 mg/kg body wt.)
E – CL1	received 100 mg/kg body wt. of ethanolic <i>Curcuma Longa</i> root extract
F- CL2	Received 200 mg/kg body wt. of ethanolic <i>Curcuma longa</i> root extract

Rats received Curcuma longa one hour before the administration of cadmium. Treatments were administered to the rats for 21 days through an orogastric tube and were sacrificed on day 22 by cervical dislocation after anaesthesia.

Table 2: Phytochemistry of *Curcuma longa* roots

Phytochemicals	Results
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Terpenes	+
Steroids	+
Cardiac glycosides	+

+ sign indicates the presence of the phytochemicals listed

treatment groups after 21 days. A significant decrease in rearing ($P<0.05$) was observed in the cadmium-treated alone group when compared to the control group and a significant increase ($P<0.05$) in the groups pre-treated with *Curcuma longa* (Cd + CL1 & Cd + CL2) when compared to the cadmium alone group (Cd). A significant decrease in ambulation ($P<0.05$) was observed in the cadmium only group when compared to the control and a significant increase ($P<0.05$) was observed in the group pre-treated with *Curcuma*

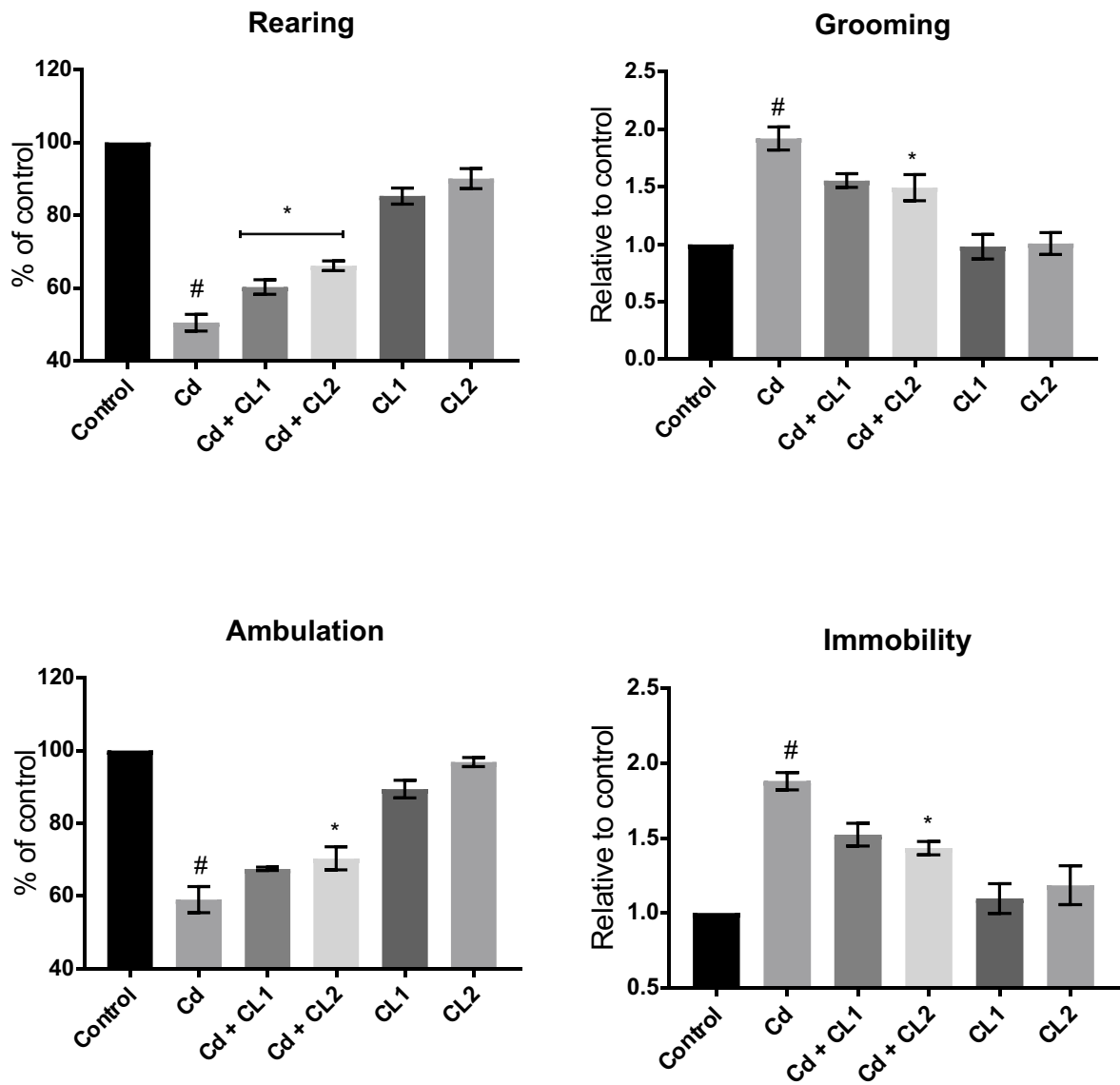


Figure 1: Open field test of control and treatment groups after 21 days. Bars represent the mean \pm SEM. [#] $p < 0.05$ compared with the control group; ^{*} $p < 0.05$ compared with the Cd-alone group

Table 3: Body weight, absolute whole brain and relative brain weights of control and treatment groups after 21 days.

Groups	Initial BW (g)	Final BW (g)	Absolute whole brain weight (g)	Relative brain weight (%)
Control	163.6 ± 3.919	177.9 ± 7.417	1.700 ± 0.058	0.960 ± 0.015
Cd	164.3 ± 6.692	151.2 ± 2.194 [#]	1.367 ± 0.067 [#]	0.900 ± 0.031
Cd + CL1	165.7 ± 9.135	164.2 ± 7.541	1.600 ± 0.100	0.980 ± 0.042
Cd + CL2	164.0 ± 6.372	166.1 ± 4.727	1.567 ± 0.033	0.947 ± 0.022
CL1	167.6 ± 4.675	177.2 ± 7.686	1.667 ± 0.033	0.943 ± 0.032
CL2	166.0 ± 3.795	177.0 ± 4.930	1.633 ± 0.033	0.923 ± 0.015

Values are given as mean ± SEM of each group. [#] p < 0.05 compared with the control group.

longa (Cd + CL2) when compared to the cadmium alone group. There was a significant increase in grooming (P < 0.05) in the cadmium alone group when compared to control and a significant decrease (P < 0.05) was observed in the *Curcuma longa* pre-treated group (Cd + CL2) when compared to the cadmium alone group. Similarly, a significant increase (P < 0.05) was observed in immobility in the cadmium alone group when compared to control and a significant decrease (P < 0.05) in the *Curcuma longa* pre-treated group (Cd + CL2) when compared to the cadmium alone group.

Effect of treatments on Antioxidant Activity

Figure 2 shows the activity of antioxidants in the cerebellum of control and treatment groups after 21 days. A significant decrease (P < 0.05) in cerebellar SOD was observed in the cadmium alone group (Cd) when compared to the control group and a significant increase (P < 0.05) was observed in groups pre-treated with *Curcuma longa* (Cd + CL1 & Cd + CL2) when compared to

cadmium-treated alone group. A significant decrease (P < 0.05) in cerebellar GPx was observed in the cadmium alone group when compared to control and a significant increase (P < 0.05) was observed in the *Curcuma longa* pre-treated group (Cd + CL2) when compared to cadmium alone group. There was a significant decrease (P < 0.05) in cerebellar CAT in the cadmium alone group when compared to control while a significant increase (P < 0.05) was observed in the *Curcuma longa* pre-treated group (Cd + CL2) when compared to cadmium alone group. A significant increase (P < 0.05) in cerebellar MDA was observed in the cadmium alone group when compared to control and a significant decrease (P < 0.05) was observed in the *Curcuma longa* pre-treated groups (Cd + CL1 & Cd + CL2) when compared to cadmium alone group.

Effects of treatment on Cerebellar histology

The activity of *Curcuma longa* on cadmium-induced cerebellar alterations across experimental groups is presented in figure 3. Plate A (control) shows normal cerebellar morphology with cells of

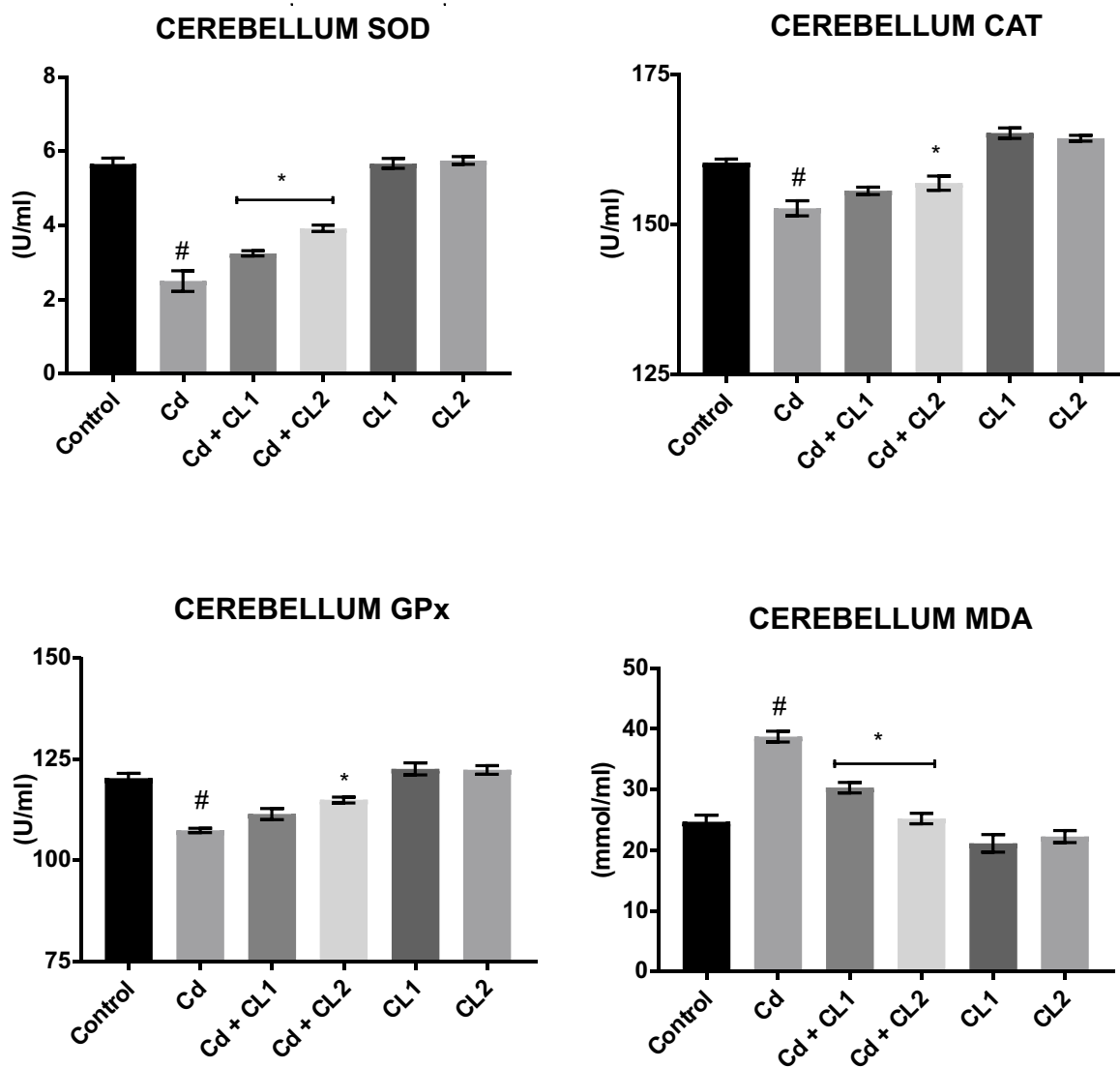


Figure 2: Activity of antioxidants in the cerebellum of control and treatment groups after 21 days. Bars represent the mean \pm SEM. # $p < 0.05$ compared with the control group; * $p < 0.05$ compared with the Cd-alone group

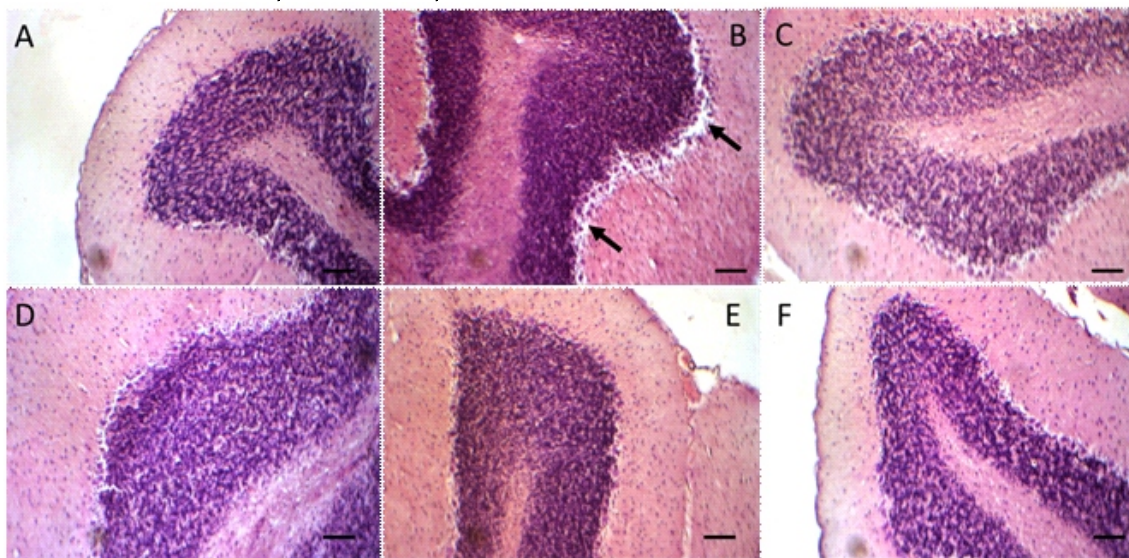


Figure 3: Effect of *Curcuma longa* (CL) on Cadmium (Cd)-induced changes in the cerebellum of experimental groups. (A) Control. (B) Cd 5mg/kg. Transverse sections show loss of Purkinje cells and gradual dissociation of Purkinje's cell layer from the granular cell layer. (black arrows) (C) 100mg/kg CL + Cd (D) 200mg/kg CL + Cd (E) 100mg/kg CL (F) 200mg/kg CL (H&E x100). Scale bar: 100 μ m

the granular, Purkinje, and molecular layers all present. In plate B (Cadmium only) loss of Purkinje cells of the Purkinje layer and visible dissociation of the Purkinje layer from the granular layer was observed. In plates C and D (*Curcuma longa* pre-treated groups), a fairly normal cerebellar morphology was observed, however, with mild loss of Purkinje cells observed in group D (Cd + CL2). In Plates E and F (*Curcuma longa* only), normal histology of the cerebellum was observed.

DISCUSSION

Cadmium, a toxic metal to which humans are constantly exposed has been reported to have various adverse effects on the body. Hence, this study aimed to investigate the protective activity of ethanolic *Curcuma longa* root extract on cadmium-

induced cerebellar damage.

The observed decrease in final body weights in rats administered with cadmium is possibly indicative of its toxicity. This agrees with findings by Orisakwe and colleagues which demonstrated that a decrease in either absolute or relative organ weight following administration of a chemical or drug is indicative of the toxic effects of that drug^[28]. Reports show that cadmium depletes glycogen (a source of energy to the brain) and weight loss, has been linked to depleted glycogen stores^[29, 30]. To this end, the loss of brain and body weights observed in the cadmium alone group may be attributed to depleted glycogen levels in the rats. Conversely, *Curcuma longa* was able to protect against the body and brain weight loss in the pre-treated groups, thus demonstrating its protective activity; this is in line with a previous study showing the protective and therapeutic effects of

herbal plants such as garlic and tomato on cadmium-induced brain and body weights loss in mice^[31].

Rearing and ambulation, classic exploratory and anxiety behaviours recorded in the open field, were significantly decreased in the cadmium alone group when compared to control. As previously reported, these findings indicate an increased level of anxiety and impairment in exploration/locomotion^[32, 33]. However, a significant increase in rearing and ambulation was observed following pre-treatment with *Curcuma longa*; this indicates that *Curcuma longa* was able to mitigate against cadmium-induced anxiety and locomotor impairment in the rats. The evaluation of grooming in rodents is considered useful for translational neuroscience research, as irregular rodent grooming can be linked to human disorders in which aberrant self-grooming is a symptom^[34]. Observational analysis of grooming activity serves as a useful measure of stress and anxiety in experimental animals^[35]. From this study, a significant increase in grooming was observed in the cadmium alone group when compared to control, thus showing increased stress levels in the rats. This corroborates reports revealing that an increase in grooming is associated with increased anxiety and stress in the rats^[34, 35].

The decrease in grooming observed in the *Curcuma longa* pre-treated groups demonstrate the stress and anxiety-attenuating activity of the extract. Immobility, a significant indicator of anxiety and lack of locomotion in rodents, was observed to be significantly increased in rats exposed to cadmium alone. This is in line with previous studies showing increased immobility in stress conditions and rats with impaired brain function^[36, 37]. Conversely, a significant reduction in immobility was observed in rats pretreated with *Curcuma longa*, thus

confirming its protective activity against cadmium in experimental rats.

The protective activity of *Curcuma longa* against cadmium-induced oxidative stress, neurobehavioral impairments, dysregulation of antioxidant activity and aberrant cerebellar morphology is possibly due to the synergetic effects of the bioactive constituents present in the plant. This observation is supported by previously identified neuroprotective roles of antioxidant supplements in the amelioration of cadmium-induced neurotoxicity^[38]. Phytochemical analysis showed that *Curcuma longa* rhizomes contain tannins, phenols, saponins, cardiac glycosides, flavonoids, terpenes, steroids and alkaloids. phenolics and flavonoids. The presence of these secondary metabolites in *Curcuma longa* may provide pharmacological and biochemical actions upon its pre-treatment to animals^[39, 40].

These findings agreed with a report that indicated the presence of phytochemicals in *Curcuma longa* rhizomes^[41, 42]. GC-MS analysis of *Curcuma longa* rhizome showed several bioactive compounds including Zingiberene, ar-turmerone, germacrone, curcumenol isocurcumenol, demethoxycurcumin and curcumin^[43]. Curcumin, a lipophilic compound and a potent scavenger of peroxy radicals, is often considered a chain-breaking antioxidant. Curcumin improves systemic markers of oxidative stress and regulates the activity of GSH, catalase, and SOD enzymes active in the neutralization of free radicals.

Other mechanisms of curcumin's activity on free radicals include its ability to scavenge ROS and reactive nitrogen species, in addition to its inhibition of ROS-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase^[17]. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione

peroxidase (GPx) are first-line defence antioxidants of the body. They collectively act against free radicals to resist their damaging effects on vital biological molecules and ultimately body tissues^[44]. Results from this study showed a significant decrease in cerebellum SOD, CAT and GPx in the cadmium alone group when compared to control, indicating increased oxidative stress. This agrees with previous reports demonstrating a reduction in the activity of the above-mentioned antioxidants following cadmium exposure^[45, 46]. It is established that high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipids^[47]. Malondialdehyde (MDA), a stable end product of lipid peroxidation can be used as an indirect measure of accumulated lipid peroxidation.

A significant increase in MDA observed in the cerebellum of rats administered with cadmium alone indicates an increased level of oxidative stress. However, in groups pre-treated with *Curcuma longa*, a significant reduction was observed in the MDA level when compared to the cadmium alone group. These findings show that *Curcuma longa* was able to protect against cadmium induced oxidative stress through proper regulation of antioxidant activity; this is in line with previous studies that show the protective effect of herbal plants against cadmium-induced oxidative stress^[48, 49]. For the histological findings, a normal cerebellar histomorphology observed in control included an intact molecular layer with its basket cells, the granular layer containing granule, brush and Golgi cells and Purkinje layer with Purkinje cells. However, in rats treated with cadmium alone, a distorted morphology of the cerebellum including loss of Purkinje cells in the Purkinje layer and dissociation of the outer Purkinje layer from the inner granular layer of the cerebellum was

observed.

This agrees with previous reports demonstrating the ability of cadmium to cause degeneration and pyknosis of Purkinje cells in the cerebellum^[50, 51]. The histology of cerebellum in *Curcuma longa* pre-treated groups show a fairly normal cerebellar microstructure with a slight loss of Purkinje cells in the Purkinje layer in the group of rats pre-treated with 100mg/kg body weight of *Curcuma longa*. These histological findings show that *Curcuma longa* was able to protect against cadmium-induced cerebellar damage and that *Curcuma longa* is not toxic to the cerebellum (seen from the histology of the cerebellum of groups E and F).

CONCLUSION

The findings of the present study demonstrate that ethanolic *Curcuma longa* root extract protected against cadmium-induced cerebellar damage and neurotoxicity in rats possibly through the potent regulation of endogenous antioxidants activity and attenuation of lipid peroxidation. These findings suggest that *Curcuma longa* could be used to develop therapeutic strategies for the prevention of neurological/neurodegenerative disorders associated with cadmium exposure.

Acknowledgement

The authors appreciate the generous assistance and contributions of Mr Etinosa Iyoha during the conduct of this research.

Conflict of interest

The authors declare no conflict of interest

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